

Supplementary material for the article:

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Supplementary material

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Overcoming hydrolysis of raw corn starch under industrial conditions with *Bacillus licheniformis* ATCC 9945a α -amylase

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Table S1. ANOVA for FFD (Response Surface Linear model)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	2400.20	3	800.07	61.92	< 0.0001	significant
X ₁ -Enzyme loading	1005.99	1	1005.99	77.86	< 0.0001	
X ₂ -Solid substrate	211.05	1	211.05	16.33	0.0024	
X ₃ -Incubation time	1183.17	1	1183.17	91.57	< 0.0001	
Residual	129.21	10	12.92			
Lack of Fit	102.38	5	20.48	3.82	0.0840	not significant
Pure Error	26.83	5	5.37			
Cor Total	2529.41	13				

The lack-of-fit test for testing the model adequacy, the best correlation between independent variables and response were carried out. High probability value of 0.084 (p-value >0.05) and low F-value of 3.82 indicated insignificant lack-of-fit of the linear model showing actually good relationship between the prediction values and responses. The predicted coefficient of determination (R^2) of 0.8657 was in a reasonable agreement with the adjusted R^2 of 0.9336, indicating a good agreement between the experimental and predicted values. A ratio between predicted and adjusted R^2 greater than 4 is desirable; ratio of 29.7 indicates an adequate experimental value (hydrolysis yield) and this model can be used to navigate the design space.

Table S2. ANOVA for CCD (Response Surface Quadratic model)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	4396.75	9	488.53	18.52	< 0.0001	significant
X ₁ -Enzyme loading	789.63	1	789.63	29.93	0.0004	
X ₂ -Solid substrate	1144.59	1	1144.59	43.39	0.0001	
X ₃ -Incubation time	2247.02	1	2247.02	85.17	< 0.0001	
X ₁ X ₂	16.96	1	16.96	0.64	0.4434	
X ₁ X ₃	4.91	1	4.91	0.19	0.6762	
X ₂ X ₃	2.39	1	2.39	0.091	0.7701	
X ₁ ²	1.19	1	1.19	0.045	0.8364	
X ₂ ²	85.33	1	85.33	3.23	0.1057	
X ₃ ²	76.69	1	76.69	2.91	0.1224	
Residual	237.43	9	26.38			
Lack of Fit	189.25	5	37.85	3.14	0.1450	not significant
Pure Error	48.18	4	12.04			
Cor Total	4634.18	18				

The Fisher *F*-test ($F = 18.52$) with a very low probability value (< 0.0001) demonstrate a high significance for the regression model. The probability (P)-values were also used as a tool to check the significance of each of the coefficients. Values of "Prob > F" less than 0.05 indicate model terms were significant and in this case X₁, X₂ and X₃ were significant model terms. The interaction effect of variables and their quadratic effect were included in the model because they were involved in an interaction and to respect the model hierarchy. The "Lack of Fit F-value" of 3.14 implies the Lack of Fit is not significant. The fit of the model was checked by the determination coefficient (R^2). In this case, the value of the determination coefficient ($R^2 = 0.9488$) indicates that only 5.12% of the total variations are not explained by the model. The value of the adjusted determination coefficient (Adj. $R^2 = 0.8975$) indicate a high significance of the model.